DECLARATION OF MICHAEL S. GERMAN UNDER 37 C.F.R. § 1.132	Attorney Docket	UCSF-129CIP
	First Named Inventor	Michael S. German
	Confirmation Number	2345
	Application Number	09/817,360
	Filing Date	March 20, 2001
	Group Art Unit	1635
	Examiner Name	Brian Whiteman
	Title: *Production of pancreatic islet cells and delivery of insulin*	

Dear Sir:

1. I, Michael S. German, declare and say I am a resident of the State of California. My residence address is 343 Glenwood Ave, Daly City, CA 94015.

2. I hold a B.A. degree in Biochemistry, which I received from the Harvard University, Cambridge MA, in 1979. I further hold an M.D. degree, which I received from the Southwestern Medical School, Dallas, TX, in 1983. I completed my residency and fellowship training at the University of Arizona Affiliated Hospitals.

3. I am an Associate Professor in the Department of Medicine and Hormone Research Institute at the University of California, San Francisco (UCSF) and Clinical Director of the UCSF Diabetes Center.

4. I have worked in the field of pancreas development for over 10 years and have published over 40 articles in the fields of pancreas development, insulin production, and transcription factors. Details of my career and publications may be found in my *curriculum vitae*, provided herewith.

5. I have been asked to a) provide information regarding my laboratory's use of neuroendocrine bHLH transcription factors to make insulin producing cells, and b) provide my opinion of the following question: would one of skill in the art would expect to be able to successfully perform the claimed *in vitro* methods using neuroendocrine bHLH transcription factors other than neurogenin3, neuroD1 and mash1.

6. It is my understanding that '360 patent application is to be viewed from the standpoint of one of ordinary skill in the art in the relevant field (a "Skilled Person") at the time of filing of the '360 patent application. The '360 patent application was filed on March 20, 2001 and relates to the field of gene expression and organ development. I would expect a Skilled Person in the relevant field of gene expression, immediately prior to and up to March 2001 (the "relevant period) to have been represented by a scientist with a Ph.D. degree and two years of postdoctoral experience. I consider that such a Skilled Person would have the ability to perform gene expression assays, and perform phylogenetic analysis of transcription factors without inventive effort.

7. Since prior to and during the relevant period I a) regularly attended external and internal meetings at which Skilled Persons presented their research, b) regularly read and reviewed scientific literature in which Skilled Persons presented their research, and c) was head of a laboratory in which several Skilled Persons have received training, I believe that I am qualified by training and experience to address what a Skilled Person would have understood from a reading of the '360 patent application.

8. The following remarks constitute the basis for my opinion that: Based on the information provided in the '360 patent application, one of skill in the art would expect to be able to successfully perform the claimed *in vitro* methods of making insulin-producing cells.

9. In my laboratory, using the methods described in the '360 patent application, we made insulin-producing cells in vitro using the class B bHLH islet transcription factors neurogenin3, neuroD1 and mash1. Data corresponding to this statement are provided herewith, in Appendix A, Appendix B, Appendix C.

10. Appendix A is a polyacrylamide gel stained for DNA products of RT-PCR experiments showing expression of insulin (the eighth panel down), in *in vitro* cultured mPAC cells (L20 and L4S2), using adenovirus vectors to express several transcription factors (e.g. ngn3, neuroD1, Mash1, and myoD). In detail, cells were treated with recombinant adenoviruses encoding LacZ or the class B bHLH proteins neurogenin3, NeuroD/BETA2, Mash 1 and MyoD. Levels of key pancreatic developmental transcription factors and islet specific protein mRNAs, e.g., insulin, were analyzed by RT-PCR 48 h after viral treatment. Tissue cultured cells or freshly isolated mouse islets were used as positive controls. PCR cycle numbers were 35-38 or 30 for the actin control.

11. Appendix B is another gel stained for DNA products of RT-PCR. When the RT-PCR experiments of Appendix A were repeated, this time increasing the number of PCR cycles, insulin production was detected in mash1 expressing cells. PCR cycle number was 38. Only results from L20 cells are shown.

12. Appendix C is a polyacrylamide gel stained for DNA products of RT-PCR experiments showing expression of insulin, in liver cells harvested from adult mice and cultured *in vitro*, using adenovirus vectors to express transcription factors (e.g. ngn3 and neuroD1). In detail, rat livers were dispersed into individual cells and the cells were separated on a size gradient. The oval cell fractions were cultured *in vitro* and treated with recombinant adenoviruses encoding LacZ or the class B bHLH proteins neurogenin3, NeuroD/BETA2. Levels of insulin mRNAs was analyzed by RT-PCR 48 h after viral treatment. Freshly isolated mouse islets were used as positive controls. PCR cycle numbers were 34 for insulin and 30 for the actin control.

13. These results establish that, using the methods described in the '360 patent application, the transcription factors neurgenin3, neuroD and Mash1, can be successfully used in methods of making insulin-producing cells in vitro.

14. With respect to the question of how representative these transcription factors are, I state the following: In my opinion, these data are sufficient to show that a number of different types of neuroendocrine class bHLH transcription factors facilitate production of insulin-producing cells in vitro. Neurogenin3, neuroD1 and mash1 are representative of the broader class of neuroendocrine class B bHLH transcription factors exemplified in Fig. 11 of the '360 patent application. As shown in Fig. 11, ngn3 is placed in the first subgroup (the neurogenins: ngn3, ngn1 and ngn2), neuroD1 is placed in the second subgroup (the neuroDs: neuroD1, neuroD2, neuroD4 and math2), and mash1 is placed in the third subgroup (the mash factors: mash1 and mash2). Since phylogenetically closely related transcription factors typically have similar functions and we have provided a single working example of each subgroup of neuroendocrine class B bHLH proteins, one of skill in the art would recognize that the

group of neuroendocrine class B bHLH transcription factors xemplified in Fig. 11. could be successfully used in the claimed methods.

15. It is therefore my opinion that one of skill in the art would expect that other members of the class of neuroendocrine class B bHLH transcription factors could be successfully used to produce insulin-producing cells in vitro.

16. I, Michael S. German, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

6/16/03
Date

Michael S. German, M.D.

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APPENDIX A

B.

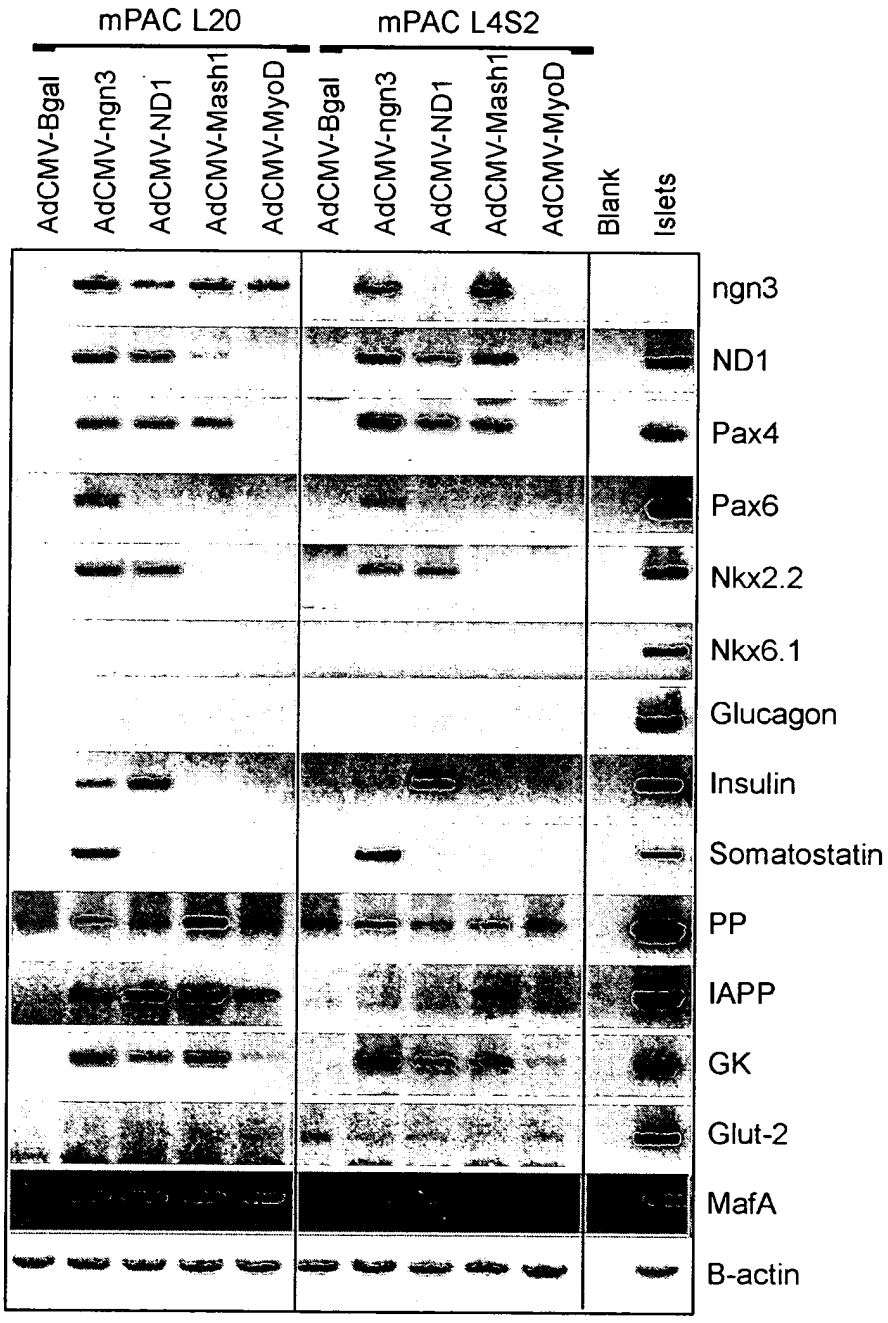
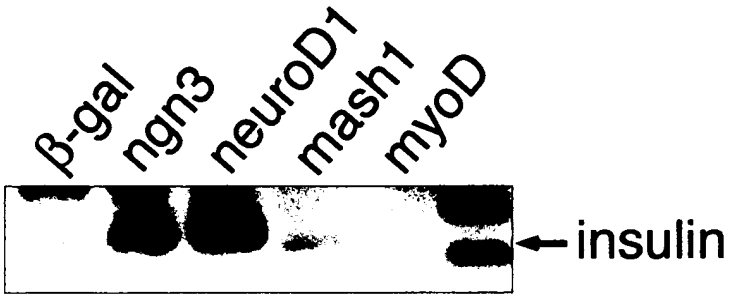
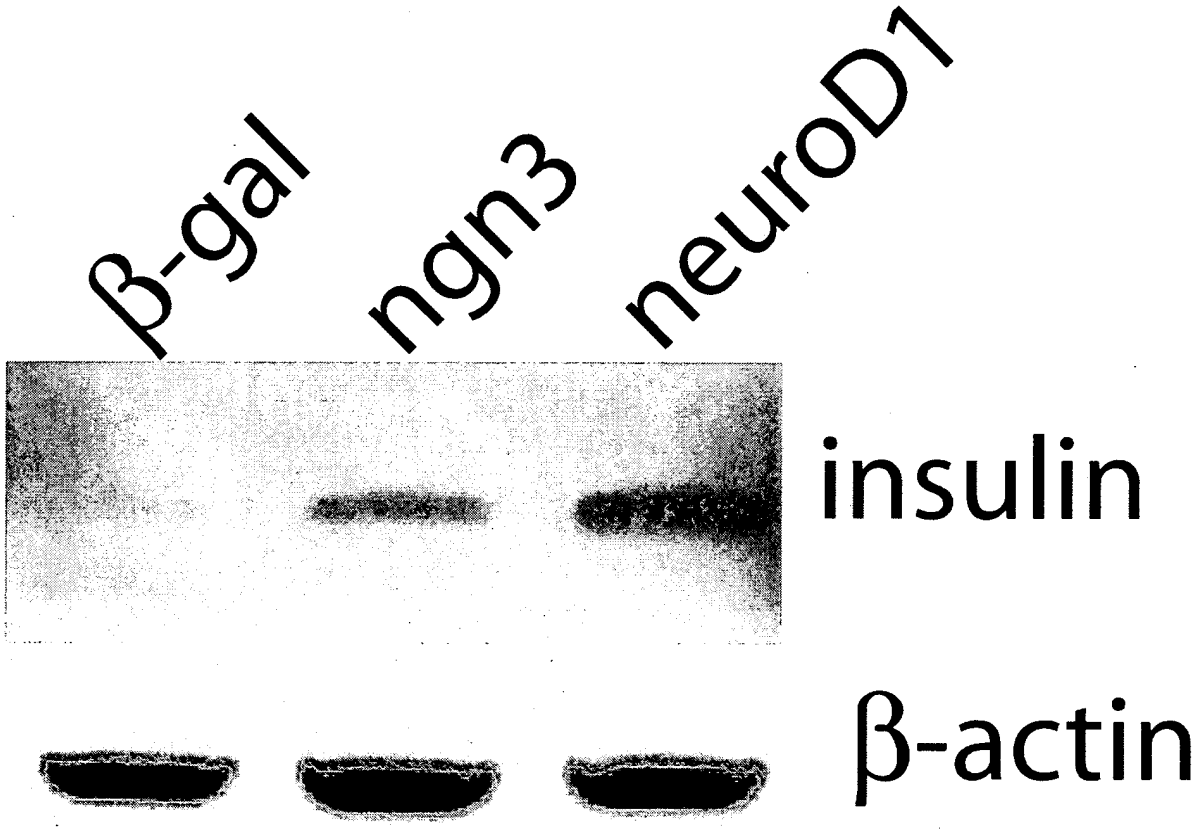


FIGURE 3B

APPENDIX B



APPENDIX C



Prepared 04/18/03

CURRICULUM VITAE

Michael Scott German

Present Address:
Home: 343 Glenwood Ave
Daly City, CA 94015

Telephone: (650) 992-9070

Work: Hormone Research Institute
University of California
San Francisco, CA 94143-0534

Telephone: (415) 476-9262
Fax: (415) 731-3612
email: german@biochem.ucsf.edu

EDUCATION

Harvard University, Cambridge, MA	B.A.	1975-79
Southwestern Medical School, Dallas, TX	M.D.	1979-83

EMPLOYMENT

1983-86	Internal Medicine Internship and Residency University of Arizona Affiliated Hospitals, Tucson, AZ
1986-87	Clinical Endocrinology and Metabolism Fellowship University of California, San Francisco, CA
1987-90	Research Fellowship Hormone Research Institute, University of California, San Francisco, CA
1990-93	Adjunct Assistant Professor of Medicine Hormone Research Institute, University of California, San Francisco, CA
1993-99	Assistant Professor in Residence Department of Medicine and Hormone Research Institute, UCSF, CA
1999-	Associate Professor in Residence Department of Medicine and Hormone Research Institute, UCSF, CA
2000-	Associate Director, UCSF Diabetes Center
2001-	Clinical Director, UCSF Diabetes Center

AWARDS

Arizona College of Physicians Clinical Vignette Award 1986.
National Institutes of Health National Research Service Award 1989-1991.
Juvenile Diabetes Foundation Career Development Award 1991-1994.
Kenneth R. Crispell Lecture, University of Virginia 2002
Kroc Lecture, University of Alabama 2003

LICENSES and SPECIALTY CERTIFICATION

California and Texas state medical licenses.
American Board of Internal Medicine certification 1986.
Endocrinology board certification 1991.

MEMBERSHIPS

Endocrine Society
American Diabetes Association
Juvenile Diabetes Research Foundation
Western Society for Clinical Investigation
American Society for Clinical Investigation

EDITORIAL BOARDS

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PUBLICATIONS

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63. Smith, S., Gasa, R., Watada, H., Wang, J., Griffen, S. and German, M. Neurogenin3 and Hepatic Nuclear Factor 1 cooperate in activating pancreatic expression of Pax4. *Submitted*

64. Ogihara, T., Watada, H., Kano, R., Ikeda, F., Nomiyama, T., Tanaka, Y., Nakao, A., German, M., Kojima, I., Kawamori, R. p38 mitogen-activated protein kinase is involved in activin A- and HGF-mediated expression of proendocrine gene neurogenin3 in AR42J-B13 cells. *J. Biol. Chem.* In press.